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Discussion

How to study SARS-CoV-2 in soils?

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ABSTRACT

Wastewater based epidemiology is increasingly being considered as a potentially useful tool for early warning about eventual new COVID-19 outbreaks. In addition, some authors are investigating on the detection and quantification of SARS-CoV-2 in sewage sludge. However, no paper has been published up to date indicating how this virus could be quantified in soil samples. In view of that, we review available data searching for methodological approaches that could guide on the quantification of SARS-CoV-2 (and even other pathogenic microorganisms) in soils.

1. Perspective and discussion

Various kinds of microorganisms have the potential to cause epidemic/pandemic diseases, and among them coronaviruses are a major concern, not just due to the current COVID-19 pandemic or previous epidemic diseases, but to the potential to generate new future outbreaks, with eventual new coronaviruses implicated (Daszak et al., 2020).. (Fig. 4)

As other viruses and other microorganisms, many different coronaviruses are continuously changing by means of mutations, and some of them have the potential for causing diseases of zoonotic transmission (Ye et al., 2020).

For SARS-CoV-2 and other different pathogenic microorganisms, the fecal-oral transmission route is a possibility that has to be considered (Heller et al., 2020; Hindson, 2020). In addition, for microorganism suffering frequent mutations, new characteristics in transmission, and the potential to be infective and cause diseases through new routes must be taken into account (Lyon and Wang, 2012; Longdon et al., 2014; Dawood, 2020).

In view of that, the detection/quantification of SARS-CoV-2 (and/or other pathogenic microorganisms) could be not just an epidemiological useful tool for wastewater based epidemiology (WBE), reporting on the incidence of the disease (Bofill-Mas and Rusiñol, 2020; Bowser, 2020; Daughton, 2020; Farkas et al., 2020a; Kitajima et al., 2020; Mao et al., 2020; Nabi et al., 2020; Núñez-Delgado, 2020a; Orive et al., 2020; Race et al., 2020; Sims and Kasprzyk-Hordern, 2020; Venugopal et al., 2020), but could inform on future risks of direct transmission, for those cases where eventual new mutations could make SARS-CoV-2 (or other

microorganisms) clearly infective by the fecal-oral route.

If this can be relevant for wastewater, it could be also for sewage sludge, and then for soils receiving the spreading of both materials, as well as for plants growing on these soils, and even for surface and groundwater in the area, which could be contaminated by means of runoff or leaching. Obviously, the risks of biotic pollution would be clearly higher in areas where wastewater and sewage sludge treatments (including disinfection) are scarce or, simply, do not take place. Further, the generation of aerosols containing SARS-CoV-2 (or other pathogenic microbes) in any of the locations where wastewater and sludge are spread, is another concern (Kitajima et al., 2020; Nghiem et al., 2020).

Taking into account that, up to now, no peer-reviewed paper focusing on SARS-CoV-2 in sewage sludge has been published, even if some comments have been presented in few peer-reviewed publications (Carraturo et al., 2020; Farkas et al., 2020b), and no peer-reviewed paper has been published dealing with the detection, determination or quantification of SARS-CoV-2 in soils (with just three papers putting together soils and SARS-CoV-2 –Lal et al., 2020; Núñez-Delgado, 2020b; Steffan et al., 2020), in this discussion piece we would propose a methodological approach in order to define some steps to follow for studying this virus (and even other pathogenic microorganisms) in soils and soil-related samples.

These studies could be: (A) In situ experiments and sampling; (B) Sampling for performing lab experiments.

(A) In situ experiments and sampling

To perform this kind of experiments and subsequent sampling, in

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selected areas affected by wastewater spreading (for example, for irrigation purposes), or receiving the application of sewage sludge (frequently used as an organic amendment), specific devices could be installed. All materials should be sterilized (or disinfected using appropriate chemical compounds when sterilization is not possible). As a reference, we will consider materials and devices previously used in some of our field researches, where microorganisms were quantified.

(i) For rather flat areas, without a slope that would favor a lateral surface or subsurface flow as runoff, liquid samples derived from soils can be sampled by means of devices that allow access to water subjected to vertical flow (descending and ascending), such as piezometers, or by means of “flow-catchers”, or even using tensiometers and portable vacuum pumps to provide the desired suction tension. These kinds of apparatus were previously described in papers where we focused on sampling and determination of both chemical and microbiological parameters, such as López-Periago et al. (2002). In fact, in these field experiments it was found that fecal bacteria remained viable in leachates after passing through up to 90 cm of soil. Some details regarding these field works can be seen in Fig. 1.

(ii) For sloped areas, also focusing on both chemical and microbiological parameters, we used troughs to sample surface runoff, and also tensiometers and catchers for vertical flow (Núñez-Delgado et al., 2002). Some details are shown in Fig. 2. In these field experiments, while just counting fecal bacteria (not viruses) we detected a prolonged persistence of viable fecal microorganisms in runoff samples generated in the pastureland where this field experiment was performed (Fig. 3).

Similar kinds of experiments and devices to those indicated above [(i) and (ii)] could be used in researches aiming to assess the degree of mobilization of viruses and/or other pathogenic microorganism of current and future concern.

After being collected, all samples must be placed on ice (or

maintained cold by other appropriate means) and transported to the laboratory in conditions that allow the survival and preservation of living microorganisms, for further quantification and eventual determination of viability.

(B) Sampling for performing laboratory experiments

In this case, sampling strategies would be defined for those areas affected by spreading of wastewater or sewage sludge, then determining the specific sites where soils would be sampled.

Soils could be sampled to obtain core structured tridimensional samples, using appropriate material (previously sterilized or disinfected), such as stainless steel cores and probes. The number of samples would be part of the overall strategy to allow the assessment of statistical significance for the results obtained. Frequently, these are surface samples, sometimes differencing among few soil depths, such as 0–20 cm, 20–40 cm. This kind of samples can be used in the laboratory to generate or extract liquid samples corresponding to the soil solution. Even, different tensions (different pressures usually expressed as specific pF unities in this case) could be used to extract liquids from porous of different diameter and with different water retention potential. All the material used must be sterilized or disinfected before use, and properly treated (sterilized/disinfected/safely-disposed) after use.

Another kind of samples can be taken by means of Edelman-type probes, without preserving the tridimensional structural integrity of the soil. They can be taken at various soil depths, frequently going from 0 to 20 cm to much more depth than in the case of core samples. Once again, the specific sites and number of samples would be defined based on a clear sampling strategy that would allow that the results obtained can be assessed as regards statistical significance. For this kind of samples it is frequent that, in certain cases, groups of subsamples are put

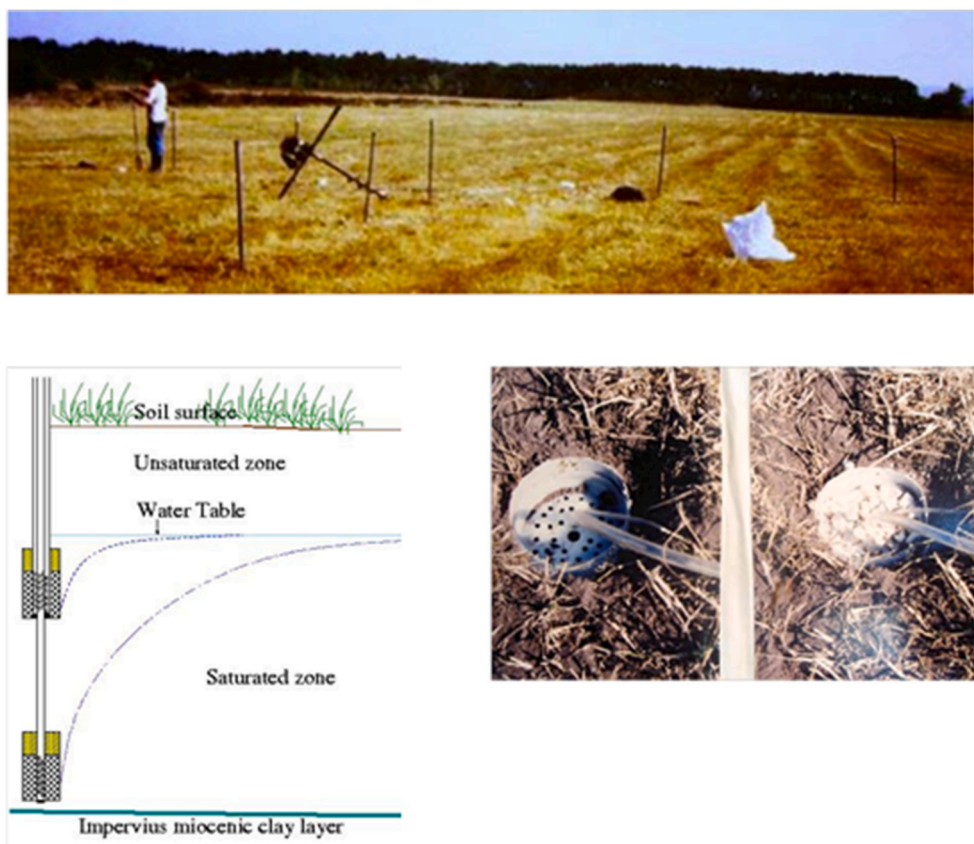


Fig. 1. Details of an experimental plot and devices used in some of our previous works in flatted areas, where samples were subjected to chemical and microbiological determinations.



Fig. 2. Experimental plot in a sloped pastureland used in some of our previous works, with details of troughs for runoff sampling, as well as of tensiometers and of flow-catchers.

together to give more reduced groups of composite samples, thus simplifying, but having incorporated the diversity and variability of the individual samples.

All samples must be handled and preserved using sterilized or disinfected material, finally putting them on ice and transporting to the laboratory for further processing.

Obviously, all samples and materials must be perfectly identified.

All researchers should wear appropriate protective equipment, and all debris generated must be packed in appropriate bags/containers for further disinfection/sterilization and eventual disposal as regulated by the local normative.

Some of these soil samples can be used in soil column experiments. In fact, and just as an example, in previous laboratory column experiments we detected that fecal bacteria remained viable in leachates after passing through 70 cm of soil (Núñez-Delgado et al., 1996). Various kinds of soils columns can be used, such as those that we described in López et al. (1998) or in López-Periáñez et al. (2000).

Also, taking into account that SARS-CoV-2 and other viruses may suffer different adsorption processes, some batch-type and stirred-flow-chamber experiments could be adapted to carry out investigations in this regard, starting from procedures such as those we have previously performed for chemical substances (Pérez-Novo et al., 2011;

Bermúdez-Couso et al., 2012; Álvarez-Esmorís et al., 2020).

At this point, in the absence of specific procedures defined for concentration, quantification and assessment of viability of SARS-CoV-2 in soil-related samples, our reflections and questions in this regard were as follows.

What is proposed or assayed for quantification of SARS-Cov-2 in sewage sludge?

What is used for other coronaviruses in soils or sludge?

What is used for other enveloped viruses?

Surrogate viruses would be needed for laboratory experiments dealing with soils when focusing on SARS-CoV-2?

Some research could be performed about diversity of soil microbiome as potential defense against SARS-CoV-2 (and/or also other pathogenic microorganisms) in soils?

As possible answers, regarding concentration and subsequent steps to finally quantify SARS-CoV-2, the procedures reviewed and commented in Farkas et al. (2020b) for water and sludge could be considered for soil-related liquid samples (lixiviates/leachates, liquids from catchers, runoff samples, soil solution), and for solid soil samples, respectively, even if more specific procedures could be assayed and further refined for the soil environment.

To take into account that the manuscripts commented by Farkas et al.

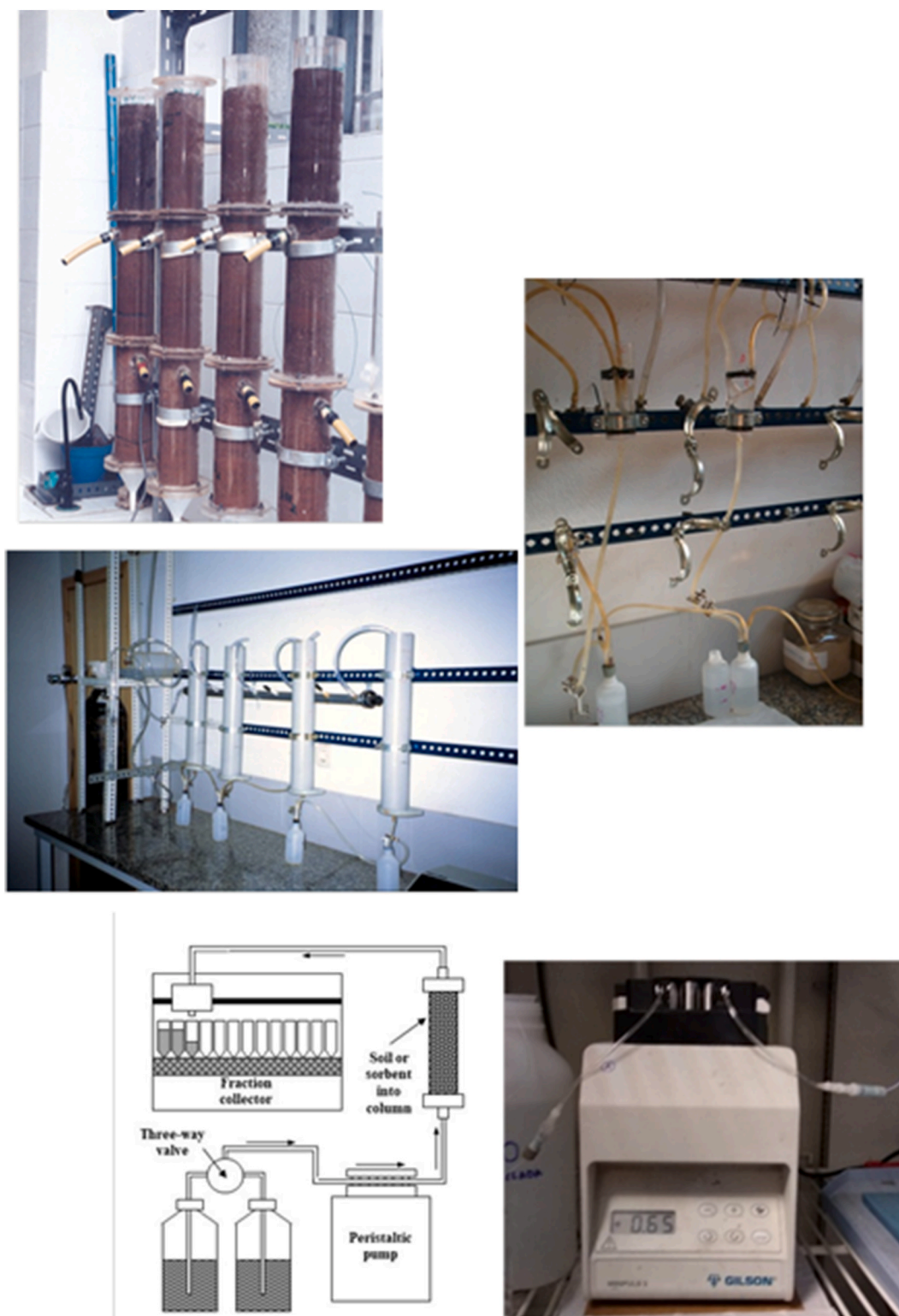


Fig. 3. Different kinds of laboratory columns used in some of our previous experiments, where both chemical and microbiological parameters were determined.

(2020b) regarding SARS-CoV-2 in sewage sludge were preprints still not subjected to peer review at the time of the publication, so, to be seen with precaution as could be not published as were at that moment. Any case, Farkas et al. (2020b) indicate that “sludge samples were either subject to RNA extraction directly, or viruses were eluted and PEG precipitated from the matrix”, and then give further details, most of them for wastewater, but overall interesting as starting point for eventual future developments of methods eventually applicable to soil

samples. Maybe, in the future, some of the preprints considered by these authors could be finally accepted and published, and then we, as all interested in performing procedures for different solid matrix (included soils), could take into account all those additional details.

In addition, we could highlight selected references dealing with other viruses in sewage sludge, namely the papers by Bibby and Peccia (2013), Nag et al. (2020) and Martínez-Puchol et al. (2020), as well as other references for papers focusing on viruses in soils, specifically

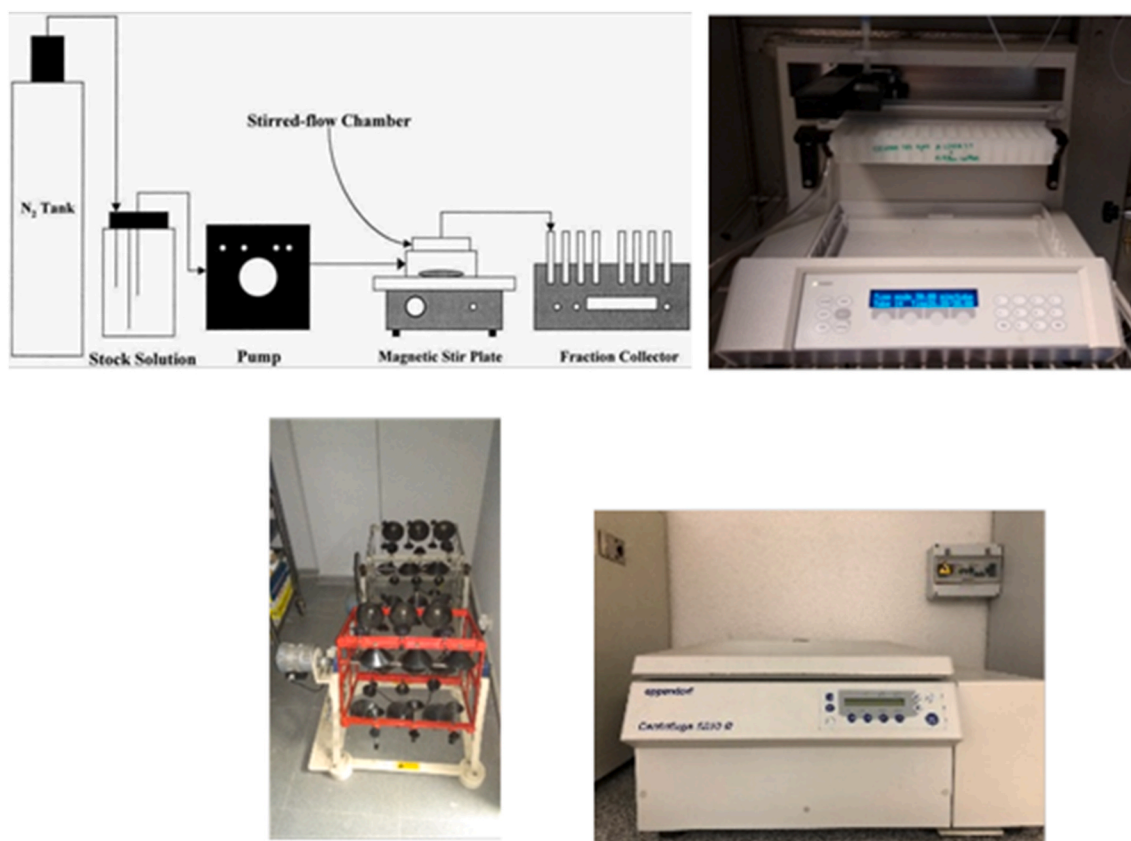


Fig. 4. Details of stirred-flow-chamber and batch-type experiments devices used in some of our previous works focused on chemical parameters.

Kimura et al. (2008), Gutiérrez and Buchy (2012) (dealing with epidemic viruses), Williamson et al. (2017), and Kuzyakov and Mason-Jones (2018). These papers would be an additional aid for those researchers trying to develop specific methods for SARS-CoV-2 and/or related viruses in samples from soil environments.

Finally, it can be taken into account that Wall et al. (2015) indicate that soil biodiversity would aid in the protection against microorganism that are pathogenic for humans and have the potential for causing epidemic outbreaks, whereas Geisen et al. (2019) emphasize on the fact that soil biodiversity should be preserved. In view of that, studies on soil biodiversity in relation to SARS-CoV-2 (and/or other pathogenic microorganisms) could be also carried out, and could be developed starting from procedures as those described in some of our previous works (Santás-Miguel et al., 2020).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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